BP203T BIOCHEMISTRY-THEORY

UNIT-TWO

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UNIT II : Carbohydrate metabolism - 10 hours

- **Glycolysis – Pathway, energetics and significance**
- **Citric acid cycle- Pathway, energetics and significance**
- **HMP shunt and its significance; Glucose-6-Phosphate dehydrogenase**
- **(G6PD) deficiency**
- **Glycogen metabolism Pathways and glycogen storage diseases (GSD)**
- **Gluconeogenesis- Pathway and its significance**

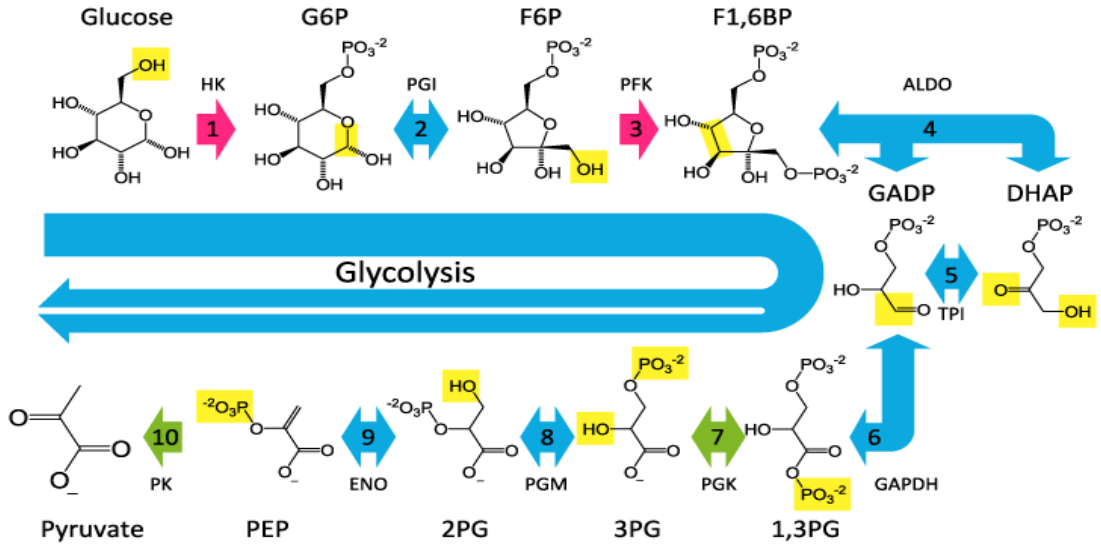
- **Hormonal regulation of blood glucose level and Diabetes mellitus**
    - **Biological oxidation**
- **Electron transport chain (ETC) and its mechanism.**
- **Oxidative phosphorylation & its mechanism and substrate**
- **phosphorylation**
- **Inhibitors ETC and oxidative phosphorylation/Uncouplers**

CARBOHYDRATE METABOLISM

Glycolysis is the metabolic process that converts glucose into pyruvic acid."

GLYCOLYSIS:

Glycolysis is the process in which glucose is broken down to produce energy. It produces two molecules of pyruvate, ATP, NADH and water. The process takes place in the cytosol of the cell cytoplasm, in the presence or absence of oxygen.

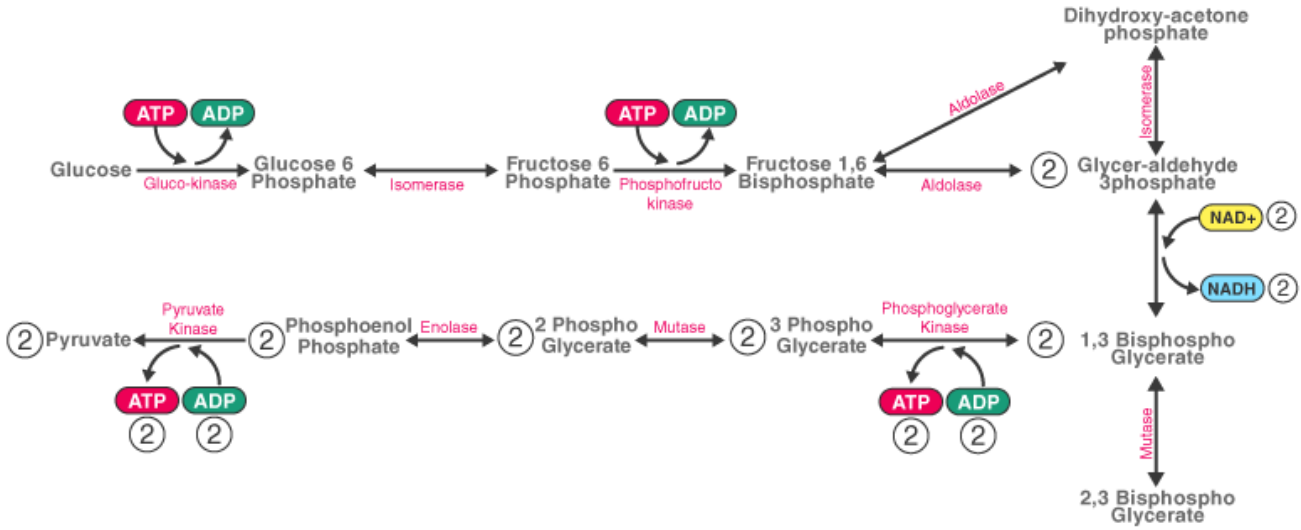


Glycolysis is the primary step of cellular respiration. In the absence of oxygen, the cells take small amounts of ATP through the process of fermentation.

This metabolic pathway was discovered by three German biochemists- Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas in the early 19th century and is known as the EMP pathway (Embden–Meyerhof–Parnas).

Glycolysis Pathway

The glycolysis pathway occurs in the following stages:



Stage 1 :

- A phosphate group is added to glucose in the cell cytoplasm, by the action of enzyme hexokinase.
- In this, a phosphate group is transferred from ATP to glucose forming glucose,6-phosphate.

Stage 2 :

Glucose-6-phosphate is isomerized into fructose,6-phosphate by the enzyme phosphoglucomutase.

Stage 3 :

The other ATP molecule transfers a phosphate group to fructose 6-phosphate and converts it into fructose 1,6-bisphosphate by the action of enzyme phosphofructokinase.

Stage 4 :

The enzyme aldolase converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which are isomers of each other.

Step 5 :

Triose-phosphate isomerase converts dihydroxyacetone phosphate into glyceraldehyde 3-phosphate which is the substrate in the successive step of glycolysis.

Step 6 :

This step undergoes two reactions:

- The enzyme glyceraldehyde 3-phosphate dehydrogenase transfers 1 hydrogen molecule from glyceraldehyde phosphate to nicotinamide adenine dinucleotide to form NADH + H^+.
- Glyceraldehyde 3-phosphate dehydrogenase adds a phosphate to the oxidized glyceraldehyde phosphate to form 1,3-bisphosphoglycerate.

Step 7 :

Phosphate is transferred from 1,3-bisphosphoglycerate to ADP to form ATP with the help of phosphoglycerokinase. Thus two molecules of phosphoglycerate and ATP are obtained at the end of this reaction.

Step 8 :

The phosphate of both the phosphoglycerate molecules is relocated from the third to the second carbon to yield two molecules of 2-phosphoglycerate by the enzyme phosphoglyceromutase.

Step 9 :

The enzyme enolase removes a water molecule from 2-phosphoglycerate to form phosphoenolpyruvate.

Step 10 :

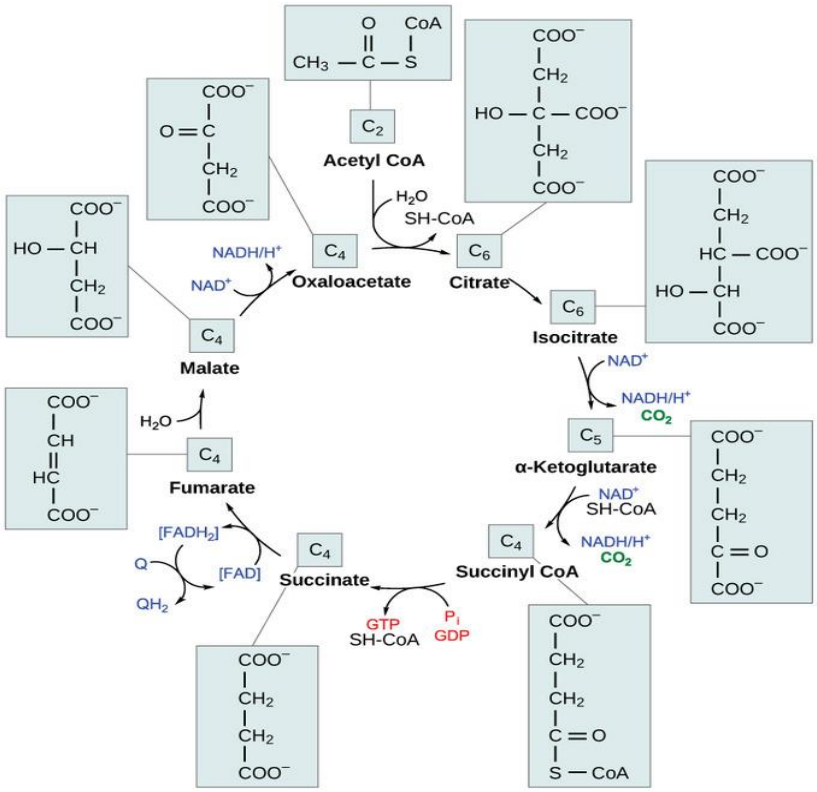
A phosphate from phosphoenolpyruvate is transferred to ADP to form pyruvate and ATP by the action of pyruvate kinase. Two molecules of pyruvate and ATP are obtained as the end products.

Key Points of Glycolysis

- It is the process in which a glucose molecule is broken down into two molecules of pyruvate.
- The process takes place in the cytoplasm of plant and animal cell.
- Six enzymes are involved in the process.
- The end products of the reaction include 2 pyruvate, 2 ATP and 2 NADH molecules.

CITRIC ACID CYCLE (KREBS CYCLE)

- The citric acid cycle takes place in the matrix of the mitochondria.
- Almost all of the enzymes of the citric acid cycle are soluble, with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion.
- Unlike glycolysis, the citric acid cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step.
- The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one GTP/ATP, and reduced forms of NADH and FADH2.
- This is considered an aerobic pathway because the NADH and FADH2 produced must transfer their electrons to the next pathway in the system, which will use oxygen.
- If this transfer does not occur, the oxidation steps of the citric acid cycle also do not occur. Note that the citric acid cycle produces very little ATP directly and does not directly consume oxygen.



The citric acid cycle: In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD+ molecules are reduced to NADH, one FAD molecule is reduced to FADH2, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the citric acid cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants.

Steps in the Citric Acid Cycle

Step 1. The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

Step 2. Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

Steps 3 and 4. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD+ to NADH. This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP. Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

Step 5. A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced.

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not

necessarily contain the most recently-added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is amphibolic (both catabolic and anabolic).

Breakdown of Pyruvate

After glycolysis, pyruvate is converted into acetyl CoA in order to enter the citric acid cycle.

Introduction

The hexose monophosphate shunt, also known as the pentose phosphate pathway, is a unique pathway used to create products essential in the body for many reasons. The HMP shunt is an alternative pathway to glycolysis and is used to produce ribose-5-phosphate and nicotinamide adenine dinucleotide phosphate (NADPH). This pathway occurs in the oxidative and non-oxidative phases, each comprising a series of reactions. The HMP shunt also has significance in the medical world, as enzyme or co-factor deficiencies can have potentially fatal implications on the affected patients.

Function

The HMP shunt is parallel to the glycolysis pathway and takes place in the cytoplasm. A 6-carbon sugar, glucose, may enter the glycolytic pathway or enter the alternative HMP shunt depending on the cell's individual needs at the time. Once the glucose enters the HMP shunt, it undergoes a series of reactions, broken down into the oxidative(irreversible) and non-oxidative phases (reversible). The oxidative phase is responsible for converting the intermediate glucose-6-phosphate to 6-phosphogluconate, using the glucose-6-phosphate dehydrogenase (G6PD) enzyme. The by-product of this reaction is the important molecule NADPH. 6-phosphogluconate then converts into ribulose-5-phosphate, and NADPH gets produced again as a by-product.[1][2][3]

The non-oxidative phase of the HMP shunt involves the conversion of ribulose-5-phosphate to ribose-5-phosphate (R-5-P) through a series of independent reactions. It is important to note that no NADPH molecules get created in this part of the HMP shunt. R-5-P in this reaction can be returned to the glycolytic pathway as fructose-6-phosphate. This step requires the transketolase enzyme with the presence of the thiamine co-factor. Thiamine also participates in a plethora of other metabolic reactions throughout the body. It is used by enzyme alpha-ketoglutarate in the Krebs cycle, for the enzyme pyruvate dehydrogenase as well as branch-chained ketoacid dehydrogenase.[3]

The HMP shunt pathway is under the regulation of the demands of NADPH in the respective tissue. The rate-limiting enzyme is G6PD and has allosteric inhibition directed by the presence of NADPH and allosteric activation via the presence of NADP+. Consequently, the activity of G6PD activity also increases in a fed state with a high carbohydrate diet, and conversely, decreases in a starving or a diabetic state.[3][4]

Mechanism

The importance of the HMP shunt is R-5-P and NADPH molecules that generated in the reaction. R-5-P undergoes a series of reactions to create the different ribose sugars that comprise the DNA and RNA molecules, required to carry genetic information. Moreover, R-5-P also converts into erythrose-4-phosphate for the synthesis of aromatic amino acids. As such, this pathway is an essential source of these ribose sugars.[5]

NADPH is also an important molecule as it has a plethora of functions throughout the body. It takes part in anabolism processes such as that of synthesis steroids and fatty acids. NADPH is also important in the respiratory burst process, an essential component of the immune response within phagolysosomes. The NADPH is used to reduce oxygen into an oxygen radical that converts into hydrogen peroxide, which in turn, transforms into bleach. Bleach in the phagolysosomes is introduced to offending pathogens to cause death. NADPH is also required to reduce the molecule glutathione. Using glutathione reductase and NAPDH, oxidized glutathione is converted into its reduced form, and can detoxify free radicals and peroxides; this limits the possibility of any free radical injury in tissues with NADPH present, namely that the red blood cells.

Since this pathway creates NADPH, a reducing agent utilized in many anabolic processes, the HMP shunt is common in more tissues than others. These tissues are those with significant anabolic functions such as adrenal cortex (fatty acid and steroid synthesis), mammary gland (milk production), liver, and red blood cells.

Clinical Significance

- Interruptions in the HMP shunt can have drastic effects on an individual. A pathology that can arise is the deficiency of the enzyme glucose-6-phosphate dehydrogenase, the enzyme required to produce NADPH in the initial step of the HMP shunt.
- The consequences are mostly seen in red blood cells as the cells can no longer defend against the constant introduction of oxidizing agents. This condition of glucose-6-phosphate dehydrogenase deficiency is an X-linked recessive disorder, most commonly seen in African Americans.
- The deficiency leads to hemolytic anemia in response to any of the offending agents such as fava beans, medications like nitrofurantoin, or anti-malarial drugs.
- Infections are the most likely offending agents to exacerbate this condition as the inflammation introduces free radicals into red blood cells that cannot detoxify those radicals, and oxidative damage ensues.
- The damage occurs as the injury causes protein and globin chains to denature within the red blood cells.
- The globin chains aggregate and form the characteristic Heinz bodies seen in G6PD deficient patients. Consequently, the spleen phagocytoses the aggregated portion of the RBC and leaves the remnant bite cells in circulation to create degmacytes, or bite cells, as seen in a peripheral smear of these patients.
- Although detrimental to the health, this condition does confer a degree of resistance to malaria as the parasites require the reduced form of glutathione to proliferate while these patients only have the oxidized form in the red blood cells.

- Thus, the evolutionary advantage maintains a deficiency in the African American population. Another use of the HMP shunt in medicine is its use in diagnosing patients with thiamine (vitamin B1) deficiency.

- Thiamine deficient patients present with a wide array of symptoms, ranging from Wernicke-Korsakoff syndrome to dry or wet beriberi. Wernicke-Korsakoff is characterized by a triad of ataxia, ophthalmoplegia, and confabulations. Wet beriberi can result in cardiac failure and dilated cardiomyopathies, while dry beriberi results in sequelae of neurological symptoms. As thiamine deficiency can lead to so many complications and potentially become fatal, it is important to detect the deficiency promptly. The deficiency is tested by administering thiamine to a suspected patient and detecting the activity of the transketolase enzyme of HMP shunt within red blood cells. If transketolase activity increases, it confirms the diagnosis of thiamine deficiency. As such, thiamine, as well as dextrose, is administered since thiamine deficiency causes impaired glucose breakdown

GLYCOGEN: THE STORAGE FORM OF GLUCOSE

Significance and localization of glucose

- Carbohydrates are key components of our diet. They provide energy, they are precursors of lipids and amino acids, and they store energy in the form of **glycogen**.

- The stored form of glucose in plants is starch, which resembles the glycogen stored in human or animal cells. The special chemical structure of glycogen allows for **rapid synthesis and breakdown**, which means that the body can respond quickly when glucose is in short supply.

- Glycogen is present in cells in the form of **cytosolic granules**. Glycogen granules contain both the enzymes for synthesis and breakdown. Glycogen is present in all cells—except in erythrocytes. The quantities used for the energy requirements of the cells themselves are minimal.

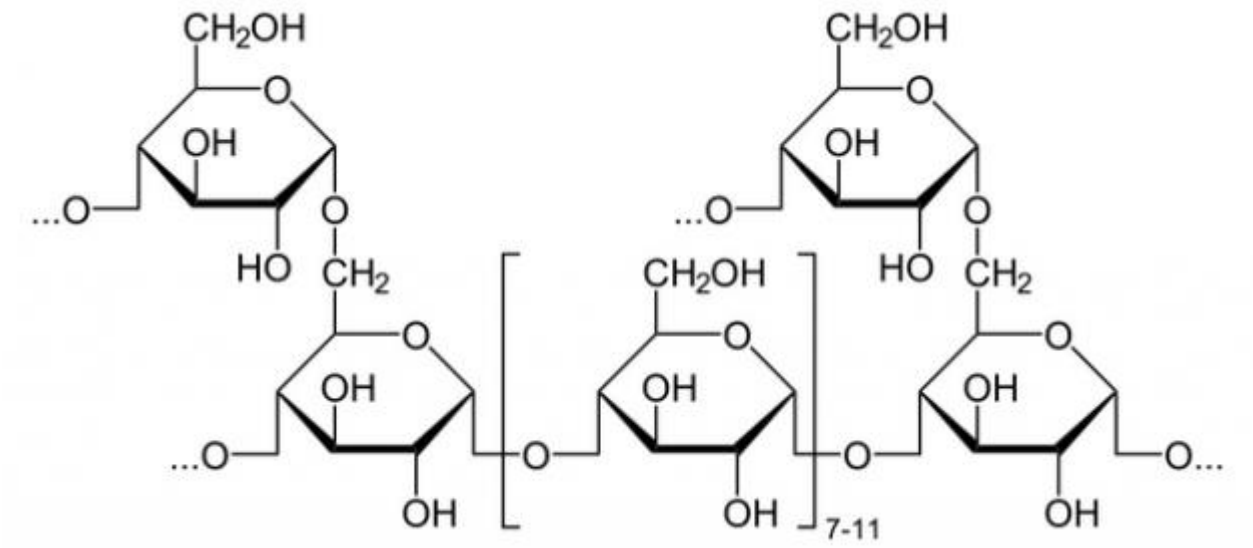
- Significant amounts of glycogen are stored in only two organs: in the liver (approximately 150 g) and in skeletal muscles (approximately 300 g).

FUNCTION OF GLYCOGEN STORAGE :

The **function of glycogen** differs greatly between the two major sites of glycogen storage—in the liver and in the skeletal muscles:

- **Liver glycogen** is the first and immediate source of glucose for the maintenance of blood glucose levels to meet the needs of the organism as a whole, especially of the brain and the RBCs.
- The **glycogen in skeletal muscles** serves exclusively as an energy source for the muscle itself.

STRUCTURE OF GLYCOGEN :



- Glycogen is a branched polymer consisting of residues of glucose, which are linked by **α-1,4 O-glycosidic** bonds with **α-1,6 branches** every 8–10 residues.
- These linkages create a tree-like polymer consisting of up to 50,000 glucose monomers, which appear as cytosolic grains when examined with an electron microscope. Glycogen provides energy storage **with minimum effect on cellular osmolarity**. It has only minimal osmotic activity due to its small size. Free glucose cannot be stored due to its high osmotic activity.
- **Note:** Free glucose would cause each cell to burst due to its osmotic activity.

- Another advantage is the branched structure of glycogen. The resulting numerous non-reducing ends ensure rapid mobilization and maintenance of the blood glucose level.

GLYCOGENESIS :

The synthesis of glycogen does not usually involve the de novo formation of glycogen but instead lengthens existing glycogen molecules by adding glucosyl residues. Every glycogen molecule has, at its core, a **glycogenin** protein, which is a glycoprotein that remains attached to the reducing end of glycogen during its degradation.

Step 1 of glycogenesis

The first step corresponds to the first reaction of glycolysis. Glucose is phosphorylated to **glucose-6-phosphate**. In skeletal muscles, this reaction is catalyzed by the enzyme **hexokinase** and, in the liver, by the enzyme **glucokinase**.

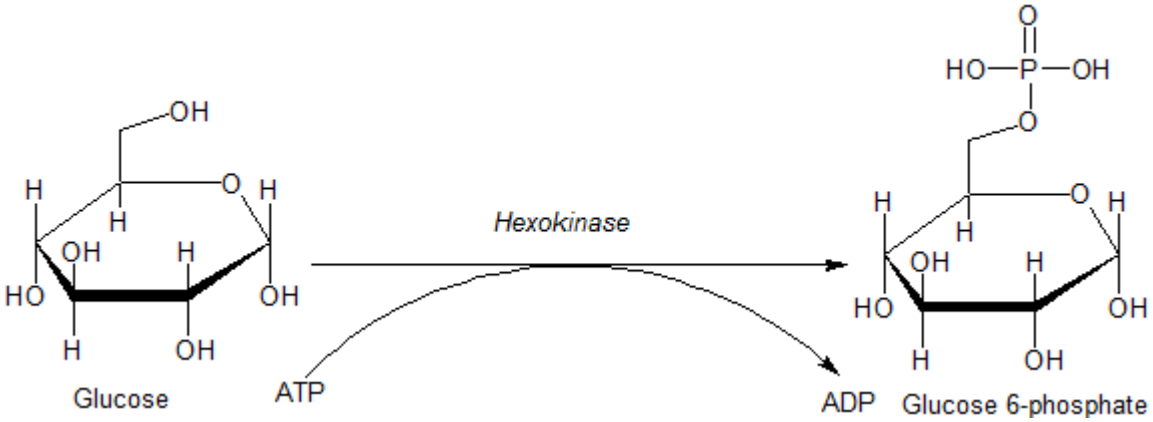


Image: Action of hexokinase with glucose as a substrate. By Jmun7616. License: Public Domain.

Step 2 of glycogenesis

The second step consists of the isomerization of **glucose-1-phosphate** by the enzyme **phosphoglucomutase**.

Step 3 of glycogenesis

In order to produce an O-glycosidic compound for the synthesis of glycogen, a high level of energy is required. For this reason, glucose-1-phosphate is initially

activated by a reaction with **uridine triphosphate (UTP)**. This produces **uridine diphosphate (UDP)-glucose** and **pyrophosphate**, which is hydrolytically cleaved into two phosphates by the enzyme **pyrophosphatase**.

Step 4 of glycogenesis

Now, the glucose can be transferred to the **C4–OH group** on one of the non-reducing ends of glycogen to form an α-1,4 glycosidic bond. This reaction is catalyzed by the enzyme **glycogen synthase**, liberating UDP, which is then recycled by conversion to UTP with adenosine triphosphate (**ATP**).

De novo synthesis of a glycogen molecule

New glucose residues can attach only to an existing glycogen molecule if the straight chain of the existing glycogen molecule is at least four glucose units long. For de novo synthesis, a **primer** (starter substrate) is essential; in this case, it is the protein **glycogenin** with its activity as a glycosyltransferase.

The **glycosyltransferase** links the **tyrosine residue** of the protein with UDP-glucose. UDP is cleaved and a glucose molecule is attached. If eight glucose units attach to the tyrosine residue of the glycogenin, the glycogen synthase can elongate the chain.

Incorporation of branch points in glycogen

The characteristic α-1,6 branches of glycogen are the products of an enzyme called amylo-(1,4→1,6)-transglycosylase = **branching enzyme**.

The **amylo-(1,4→1,6)-transglycosylase** adds branches to the growing glycogen molecule by forming α-1,6 linkages by binding to a linear α-1,4 chain that consists of at least 11 **glucose monomers**. Of these, a chain of seven glucose monomers is removed and transferred to the OH group of the C6 of a glucose residue. Thus, located between two branch points are at least four glucose monomers.

Note: The high density of branches characteristic for glycogen gives rise to a great number of non-reducing ends, which determines the possible rates of synthesis and breakdown and enables a maximum speed of glucose release. It is

thus possible to quickly access glycogen stores to lower or increase blood glucose levels when required.

THE BREAKDOWN OF GLYCOGEN: GLYCOGENOLYSIS :

- **Glycogenolysis** follows a different pathway than glycogenesis.

- **Glucose-1-phosphate**, a high-energy compound, is released. The steps of glycogenolysis are as follows:
- At the free non-reducing ends of glycogen, **glycogen phosphorylase** catalyzes phosphorolytic cleavage of the α-1,4-glycosidic linkages of glycogen, releasing glucose-1-phosphate as the reaction product. For this purpose, free inorganic phosphate is required.
- Glucose-1-phosphate can isomerize to glucose-6-phosphate and enter glycolysis.
- The liver enzyme **glucose-6-phosphatase** converts glucose-6-phosphate to glucose, which regulates and maintains blood glucose levels. The skeletal muscles lack this enzyme and can therefore not release glucose into the bloodstream.
- Glycogen phosphorylase is **dependent on pyridoxal phosphate (PLP)** and can only cleave α-1,4-glycosidic bonds. It stops cleaving α-1,4 linkages four glucose monomers away from an α-1,6 branch point.

DEGRADATION OF BRANCHING POINTS DURING GLYCOGENOLYSIS :

The **debranching enzyme** (**4-alpha-glucanotransferase**) is a bifunctional enzyme that is responsible for the degradation of branching points. It has the following functions:

- **Transferase activity:** The enzyme 4-alpha-glucanotransferase transfers a segment of three glucose units from α-1,6 branched four-unit chains (the result of glycogen phosphorylase activity) to an adjacent branch of the glycogen chain.

- **Glucosidase activity:** The enzyme 4-alpha-glucanotransferase hydrolytically cleaves the remaining α-1,6 linkage, producing glucose and a linear chain of glycogen.

Regulation of Glycogen Metabolism

- Glycogen metabolism is regulated by two enzymes: **glycogen phosphorylase** and **glycogen synthase**. The coordination is mainly dependent on hormone-mediated and, in part, allosteric regulatory effects.
- The **allosteric regulation** is a form of regulation of enzyme activity carried out by certain enzymes (**allosteric enzymes**) that are almost always composed of multiple subunits. These may occur in more than one stable conformation.
- A negative feedback mechanism leads to the inhibition of activity or synthesis of one or more enzymes through the end product. The inhibition of enzyme synthesis is called **enzyme expression**. The inhibition of enzyme activity is called an allosteric effect.